Painting analysis of chromosome aberrations induced by energetic heavy ions in human cells

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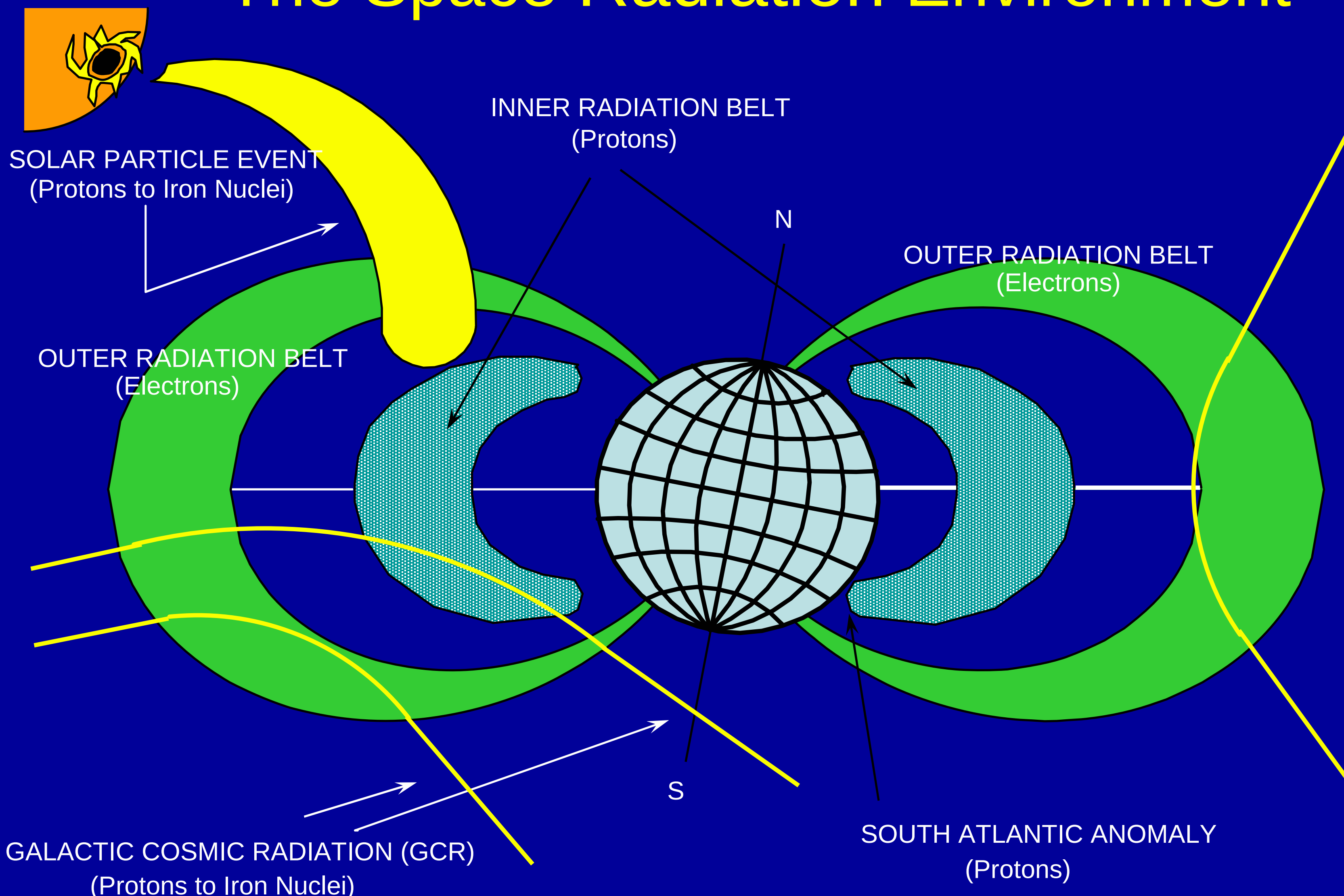
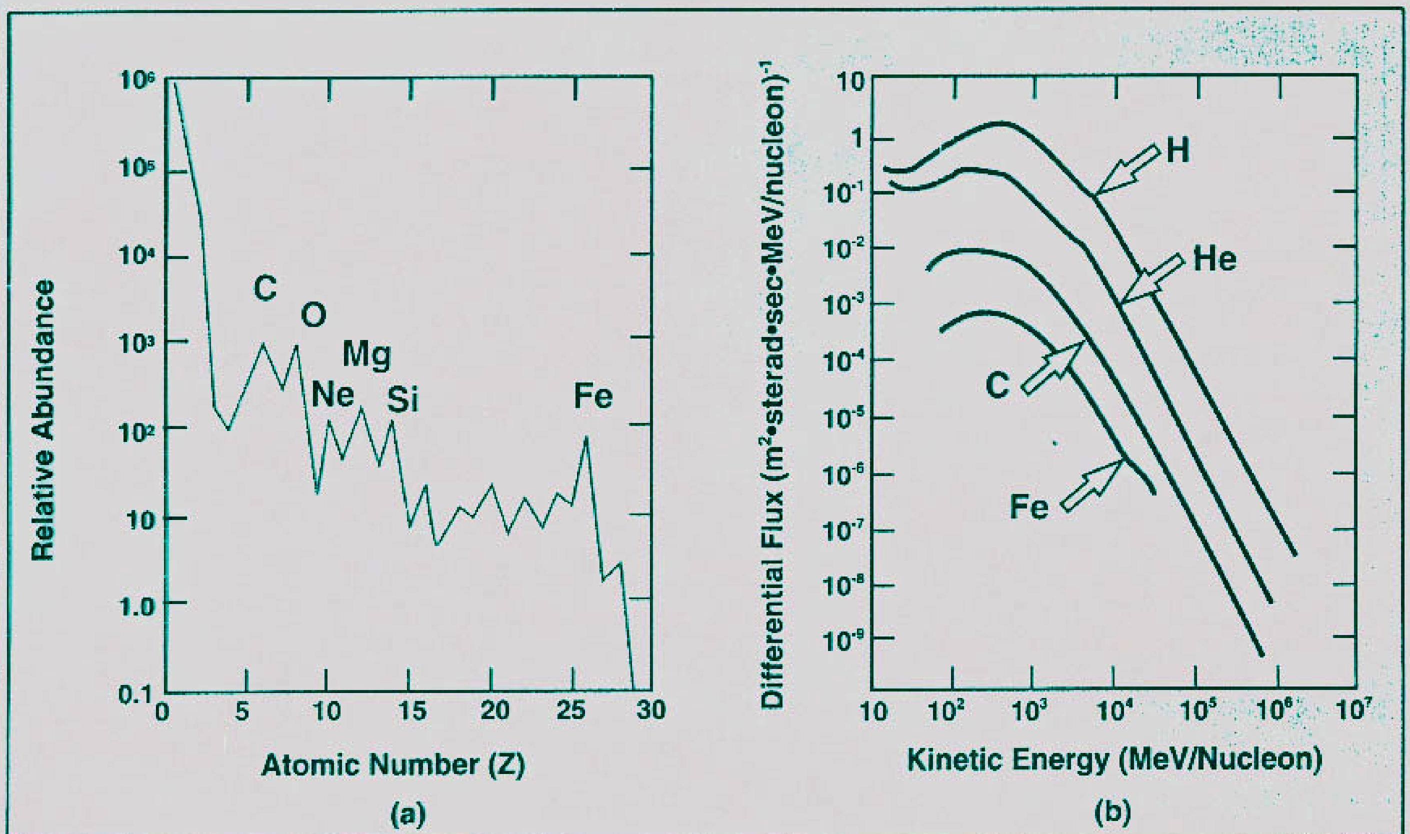
The Space Radiation Environment



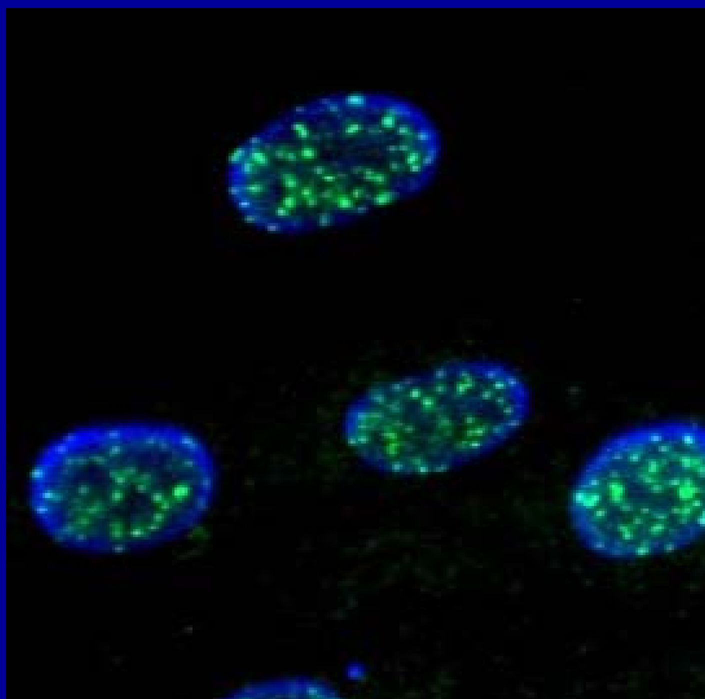
Representation of the major sources of ionizing radiation of importance to manned missions in low-Earth orbit. Note the spatial distribution of the trapped radiation belts.

Galactic cosmic radiation

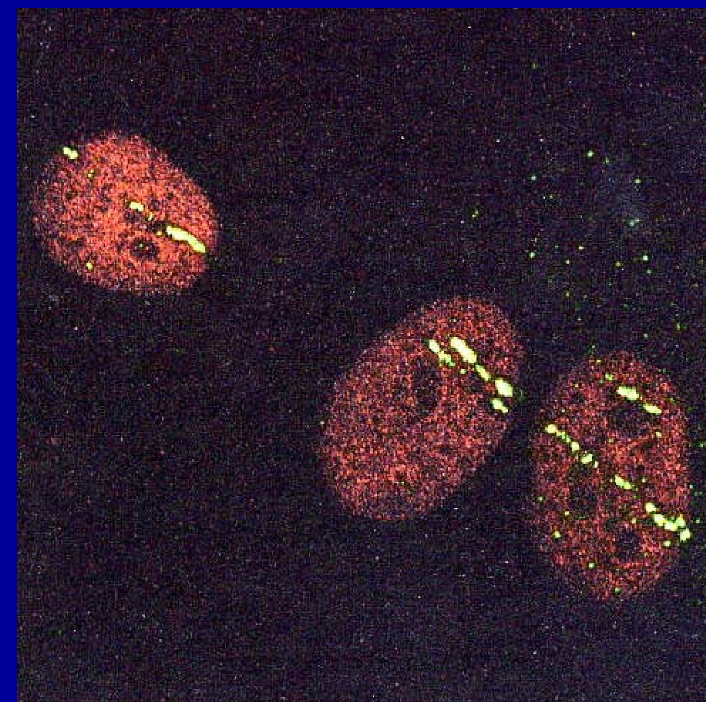
Figure D.1. Abundances (a) and Energy Spectra (b) of GCR



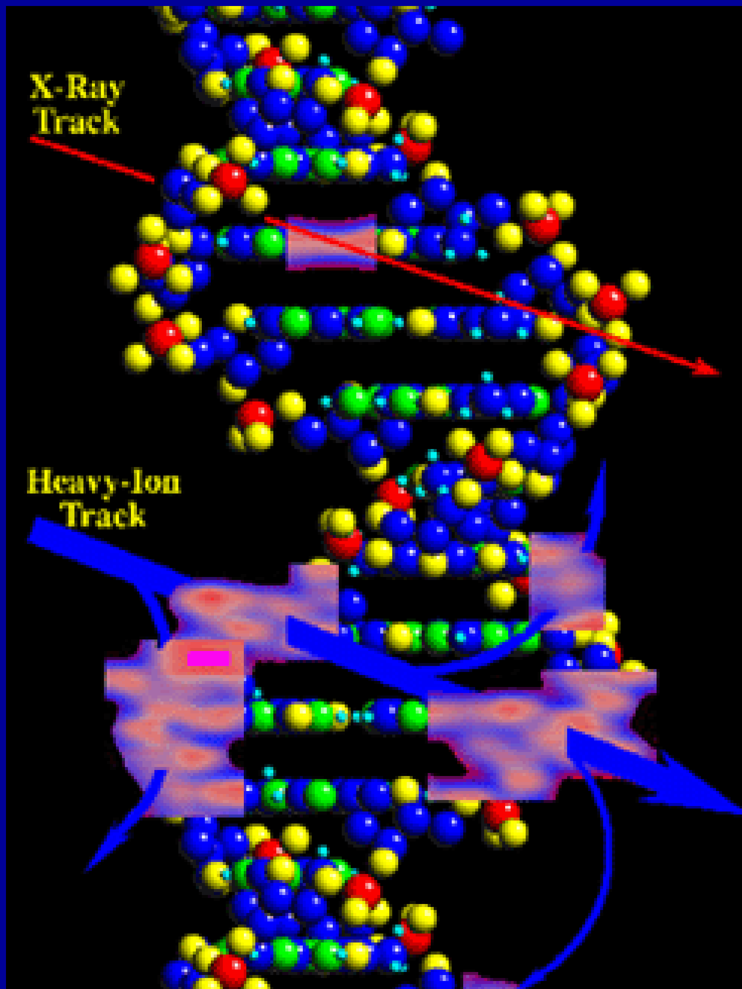
DSB induction

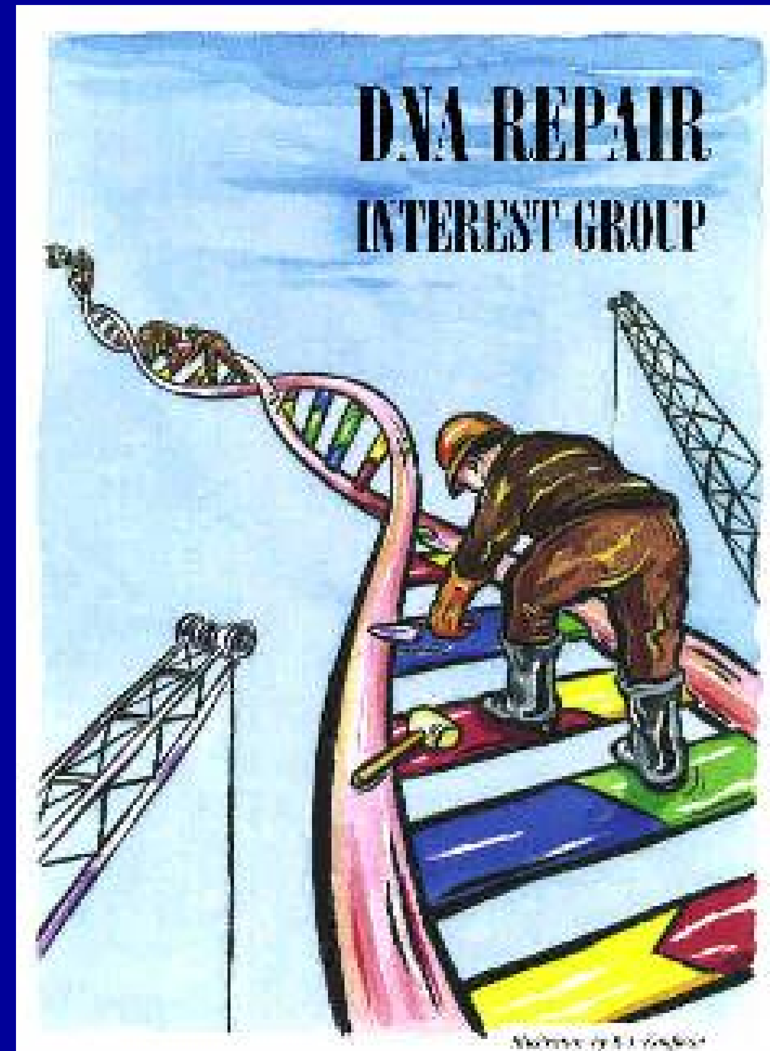


Low-LET

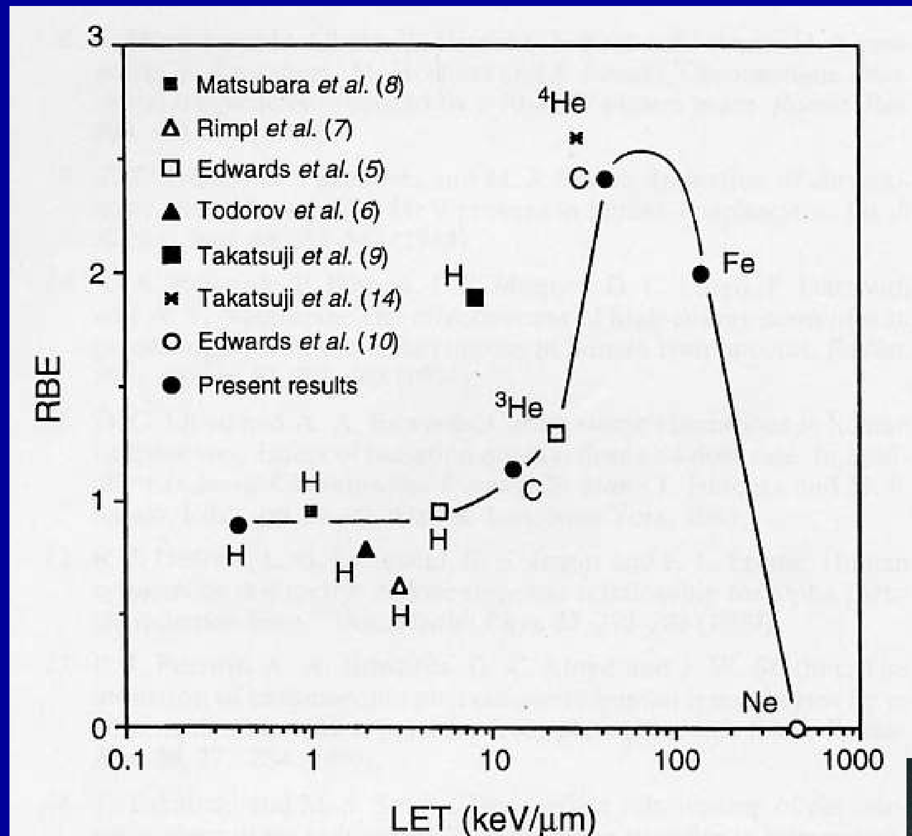


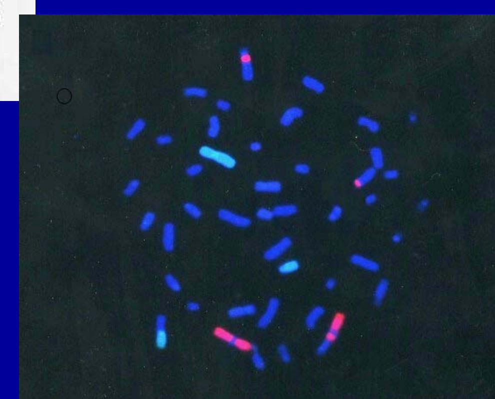
High-LET


X-Ray
Track
Heavy-Ion
Track


DNA REPAIR
INTEREST GROUP

Radiation-induced chromosome aberrations in lymphocytes in vitro





Wu, Durante, George and Yang, *Radiat. Res.* (1997)

Why do we study chromosomes?

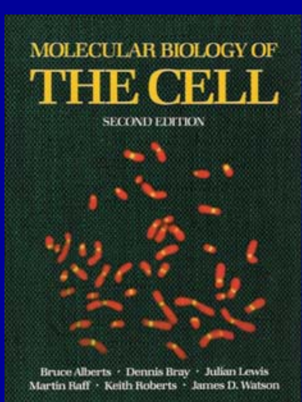
Chromosome aberrations in astronauts' lymphocytes are analyzed to determine the biological dose received from long-term space missions

Biodosimetry results

Subject	TLD reading (cGy)	Biological dose measured using values for translocation (cSv)	Average RBE*
1	3.06	16	4.2
2	3.78	18	3.8
3	5.68	20	2.8
4	4.16	23	4.4
5	4.16	14	2.7
6	4.16	12	2.3

*25% correction for high-LET radiation in TLD measurement is included.

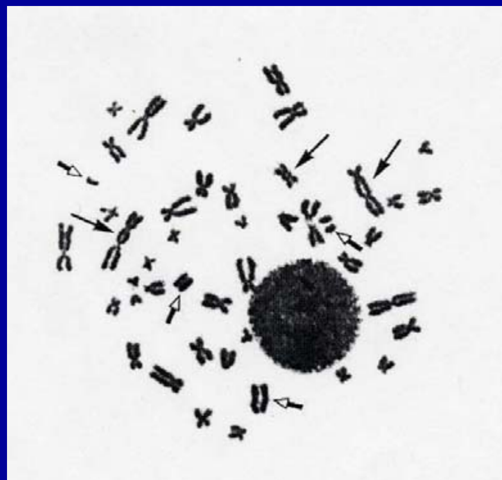
Mission duration: 3-5 months
Altitude: 190 NM
Inclination: 51.6 degree



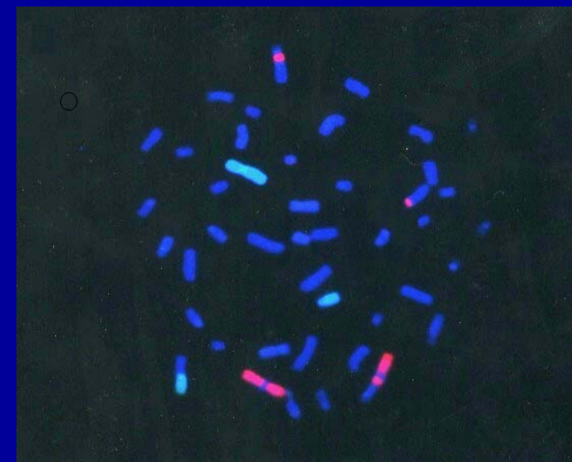
Objectives

- Are there bio-signatures for space radiation exposure?
- Are chromosome aberrations associated with radiation risks?

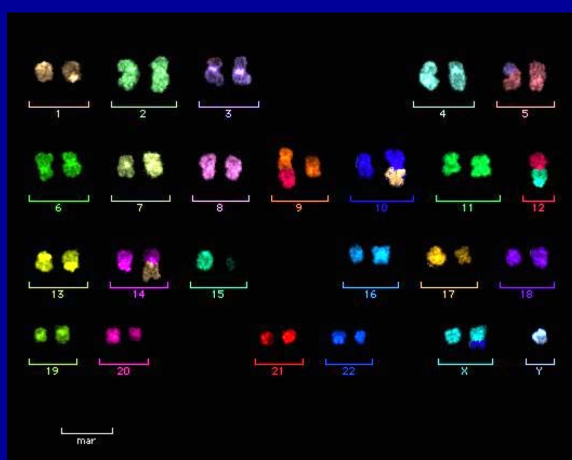
Chromosome staining/painting techniques



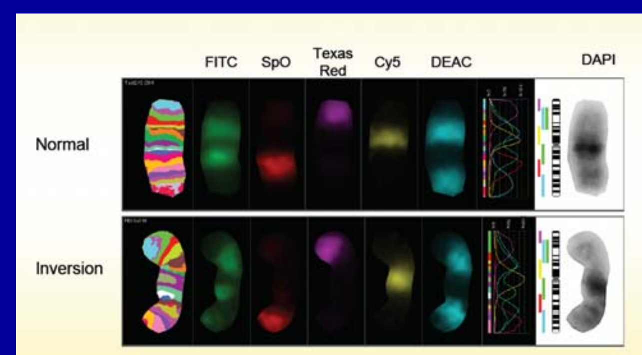
Giemsa



FISH



mFISH

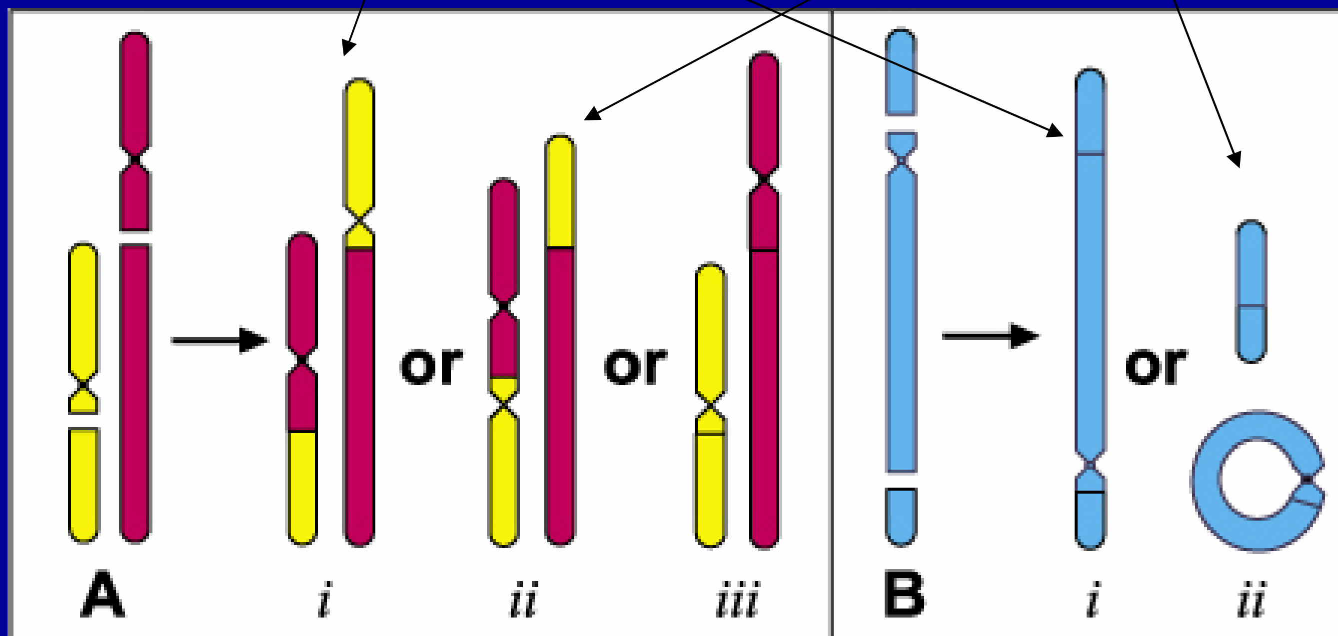


mBAND

Chromosome aberration

Stable

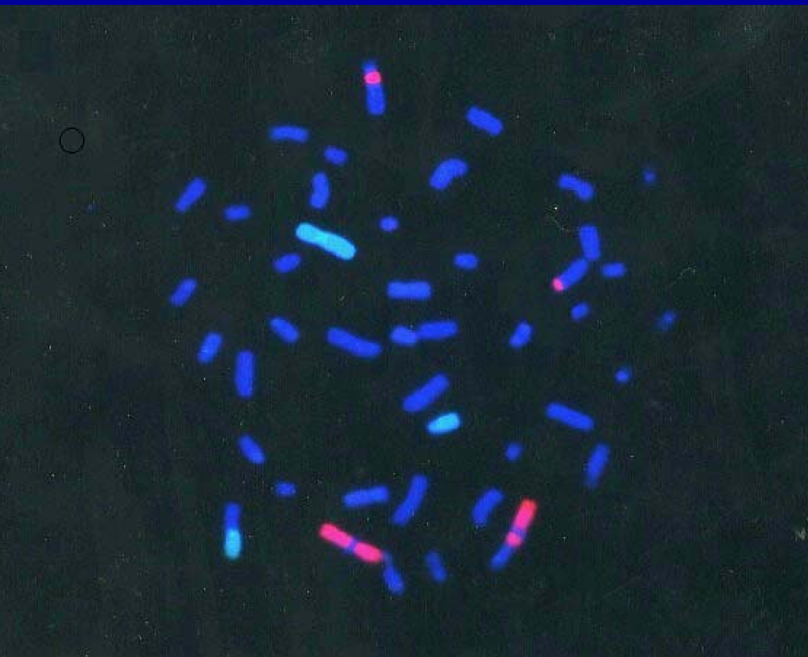
Unstable

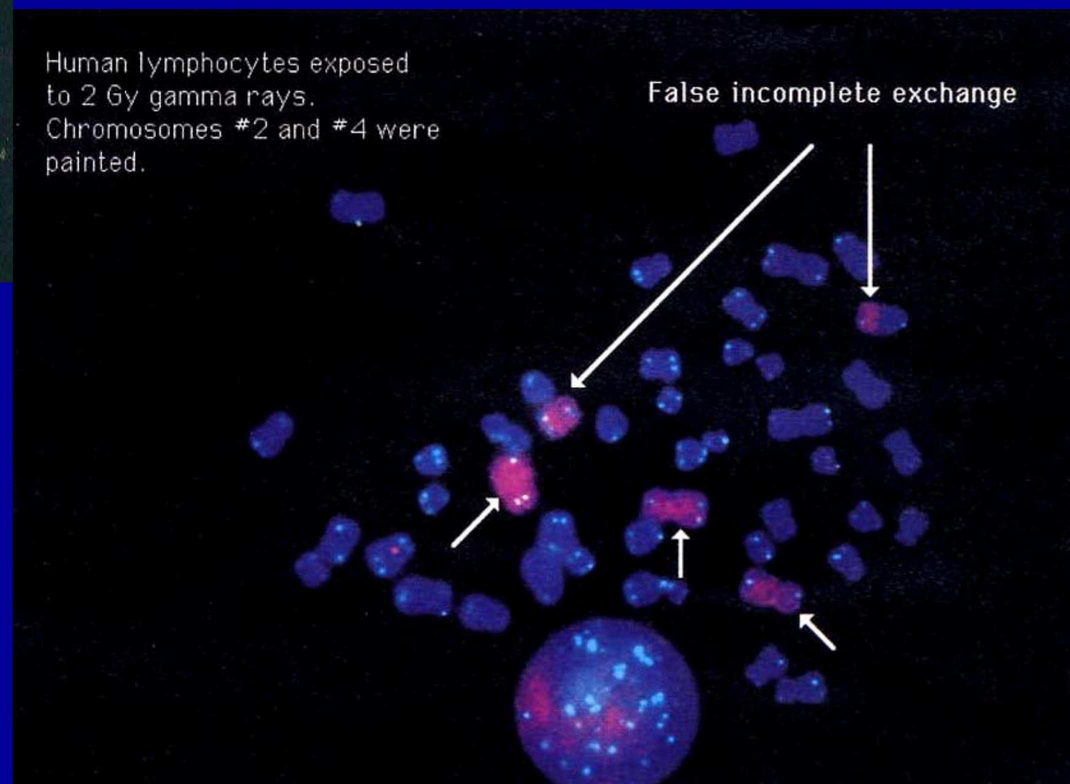


Inter-chromosome exchange

Intra-chromosome exchange

Telomere Analysis





Truly incomplete exchanges in human lymphocytes exposed to gamma rays in vitro

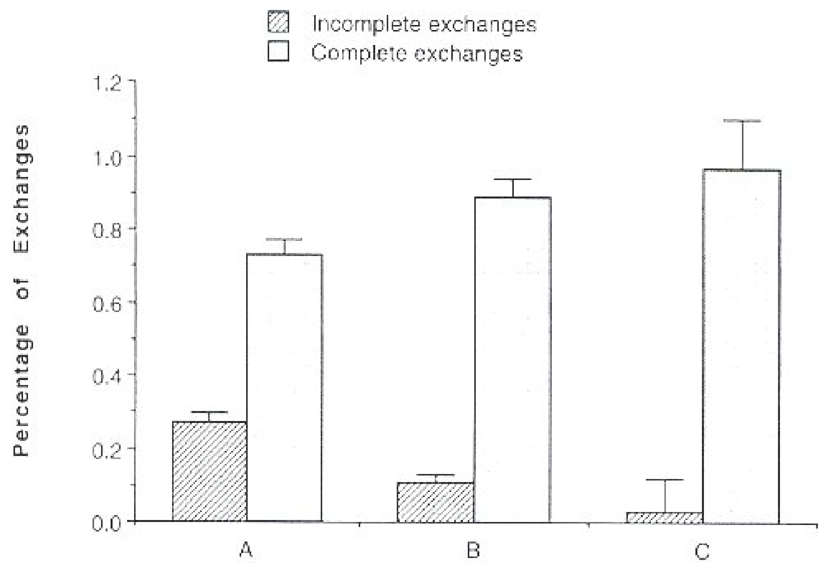
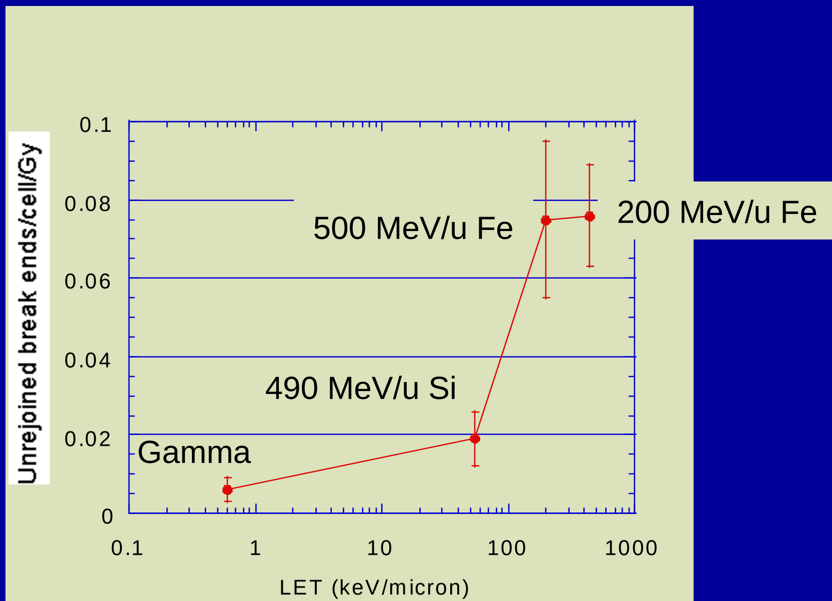


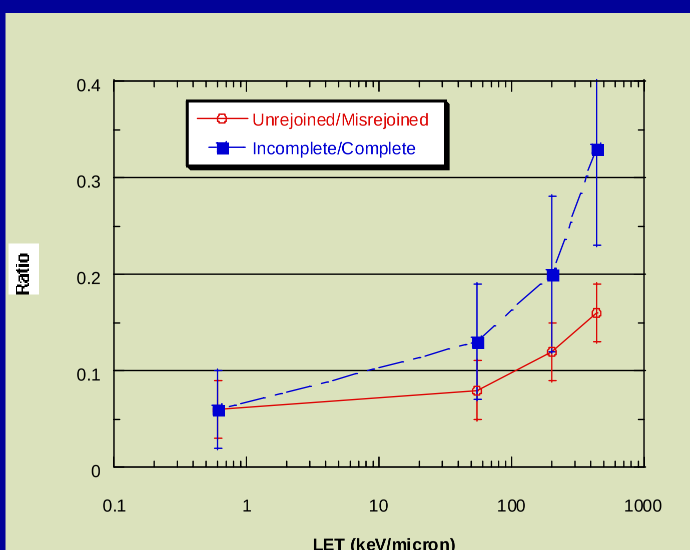
Figure 4. Percentage of complete and incomplete exchanges from the sum of the data. (A) The percentage of incomplete exchanges was 27% without the consideration of telomere probes. (B) With false incomplete exchanges included as complete, the percentage of incomplete exchanges decreased to 11%. (C) The estimated percentage of true incomplete exchange was 3%. (bar = 1 SD)

Most of the incomplete exchanges analyzed with FISH are actually complete

Wu, George and Yang, IJRB (1998, 1999)

Human fibroblast cells exposed to radiation of different qualities



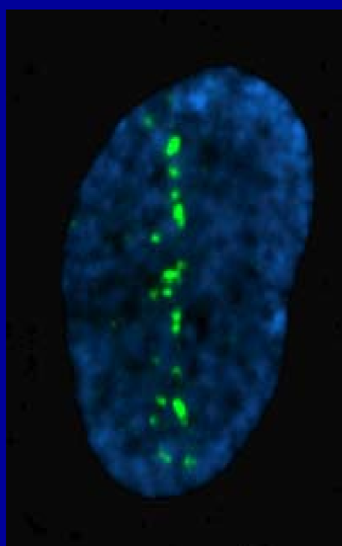


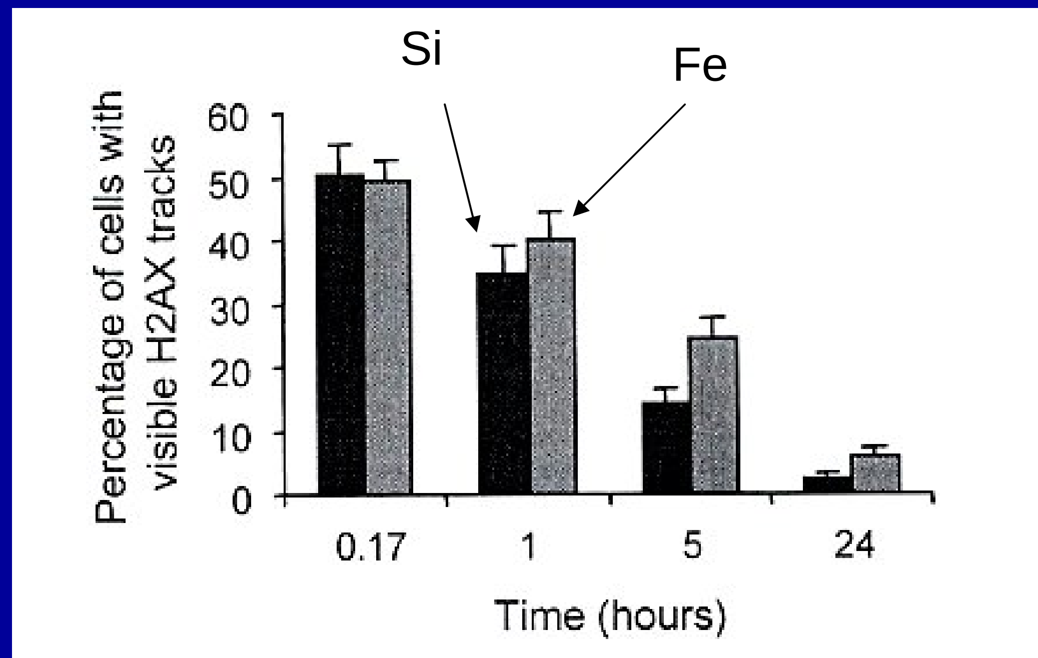
- The fraction of unrejoined chromosome breaks are higher for high LET

- Unrejoined breaks and incomplete chromosomal exchanges are possible biosignatures of high-LET radiation

Wu, Durante, Furusawa, George, Kawata and Cucinotta, Rad. Res. (2003)

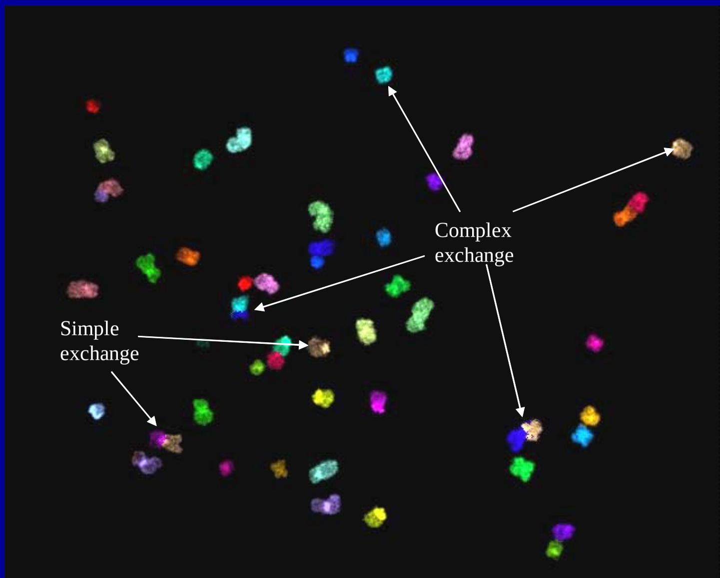
High-LET radiation induces more unrejoined DNA double strand breaks

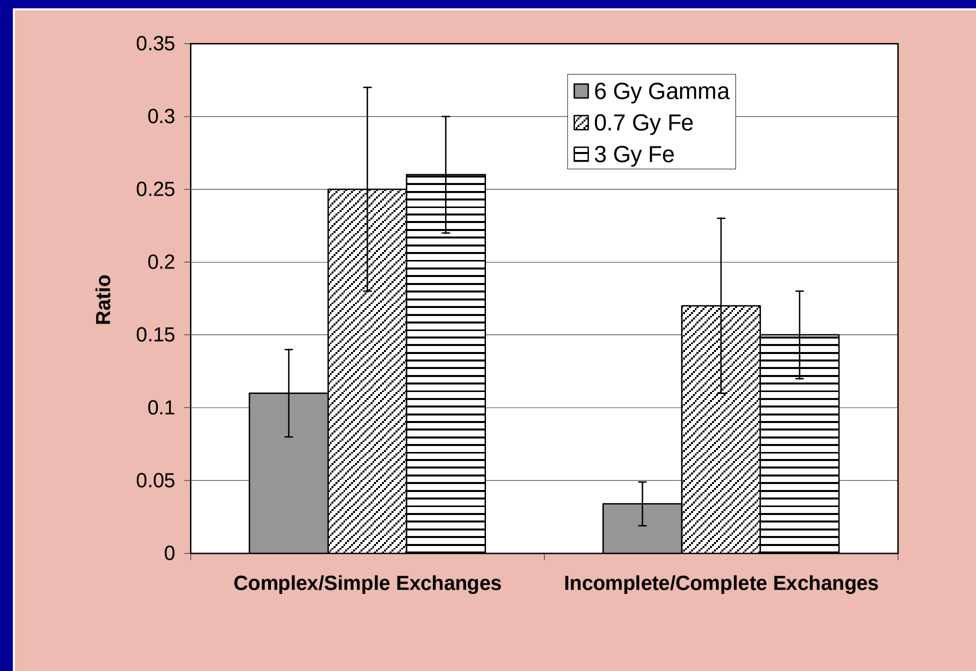




Desai, Davis, O'Neill, Durante, Cucinotta and Wu, Rad. Res. 2005

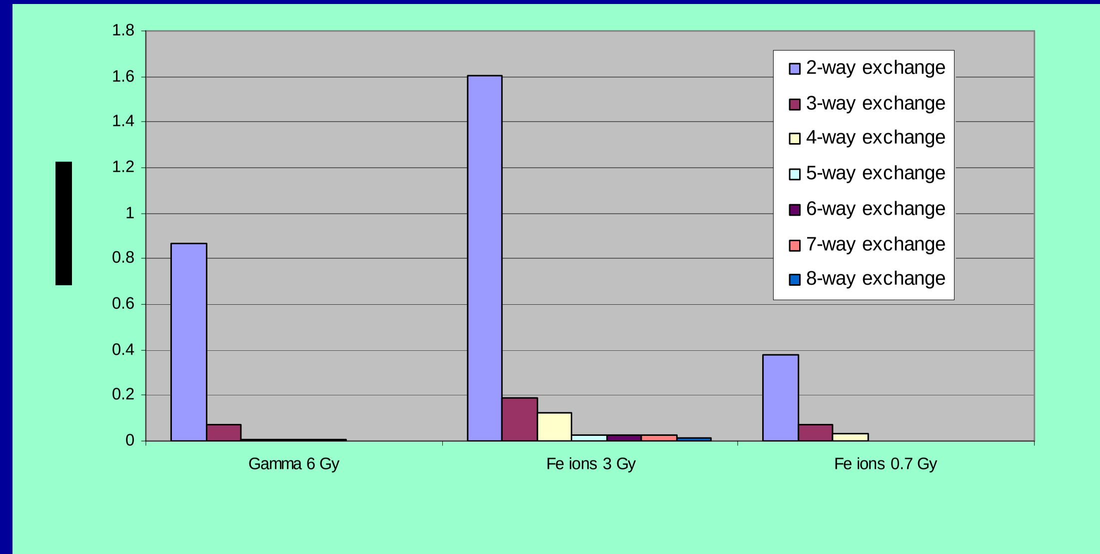
Complex aberrations -- mFISH analysis

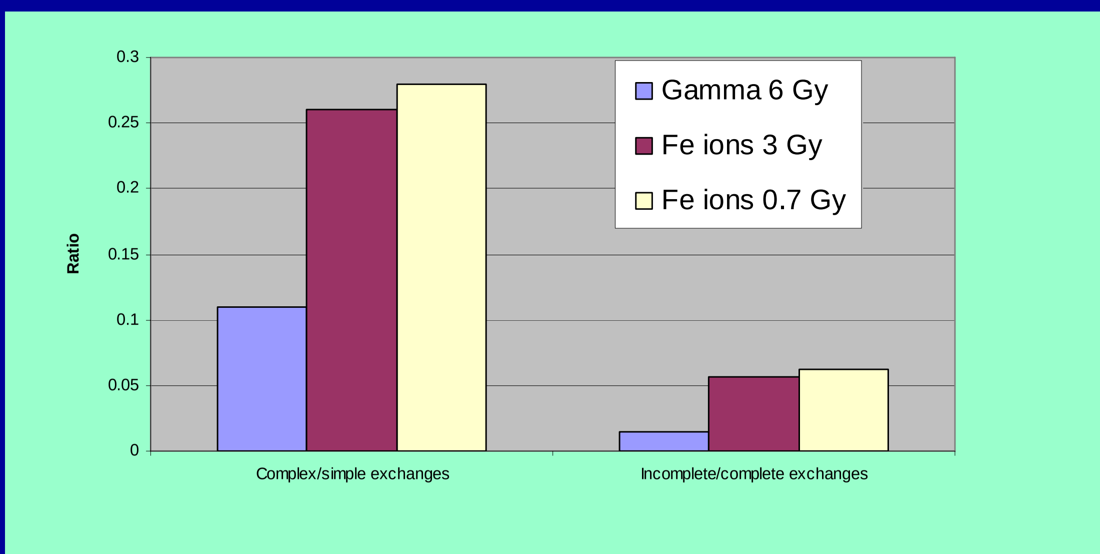




Wu, Durante, Furusawa, George, Kawata and Cucinotta, Adv. Space Res. (2002)

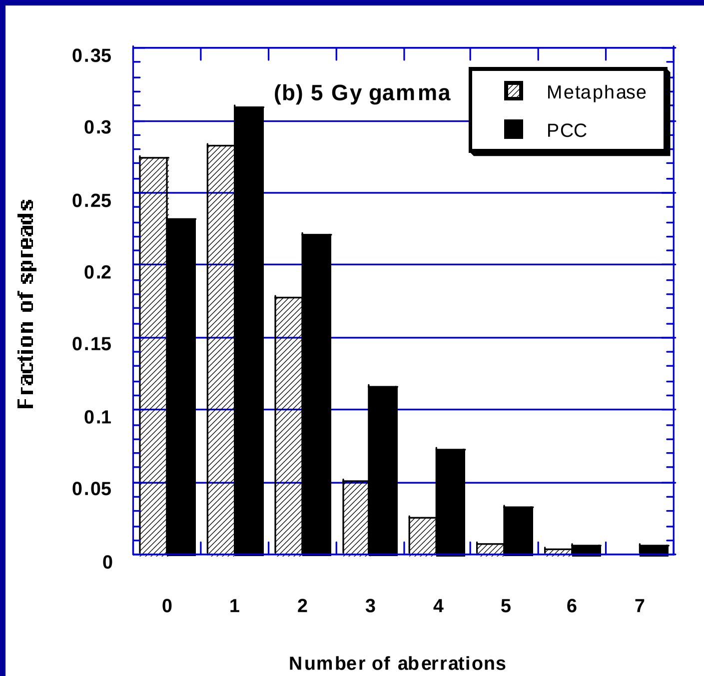
Complex type aberrations





mFISH showed a higher fraction of complex and incomplete exchanges for high-LET

Interphase vs. metaphase: Issues of biosignature
(F ratio: Ratio of dicentrics to centric rings)

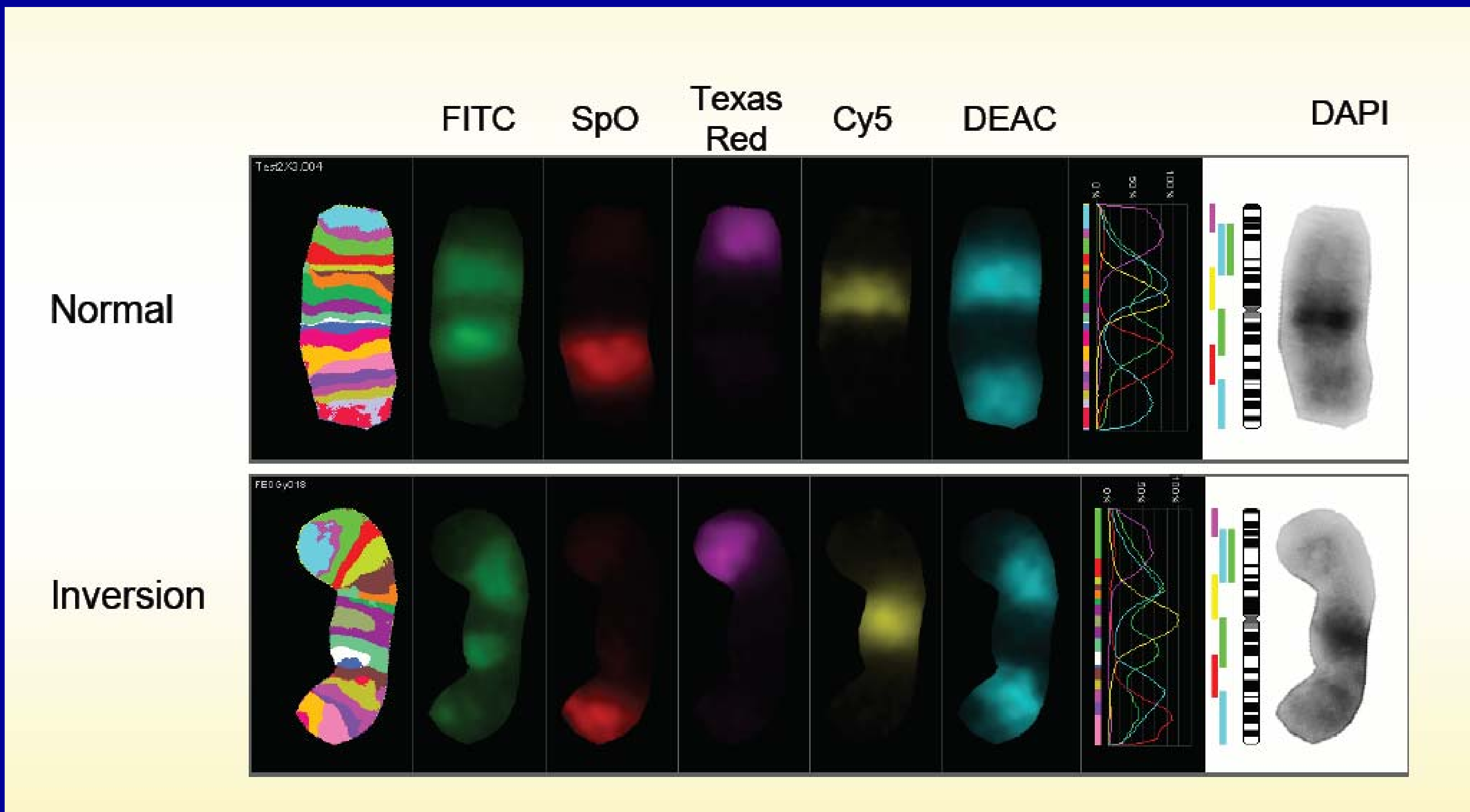


Centromere probes were used.

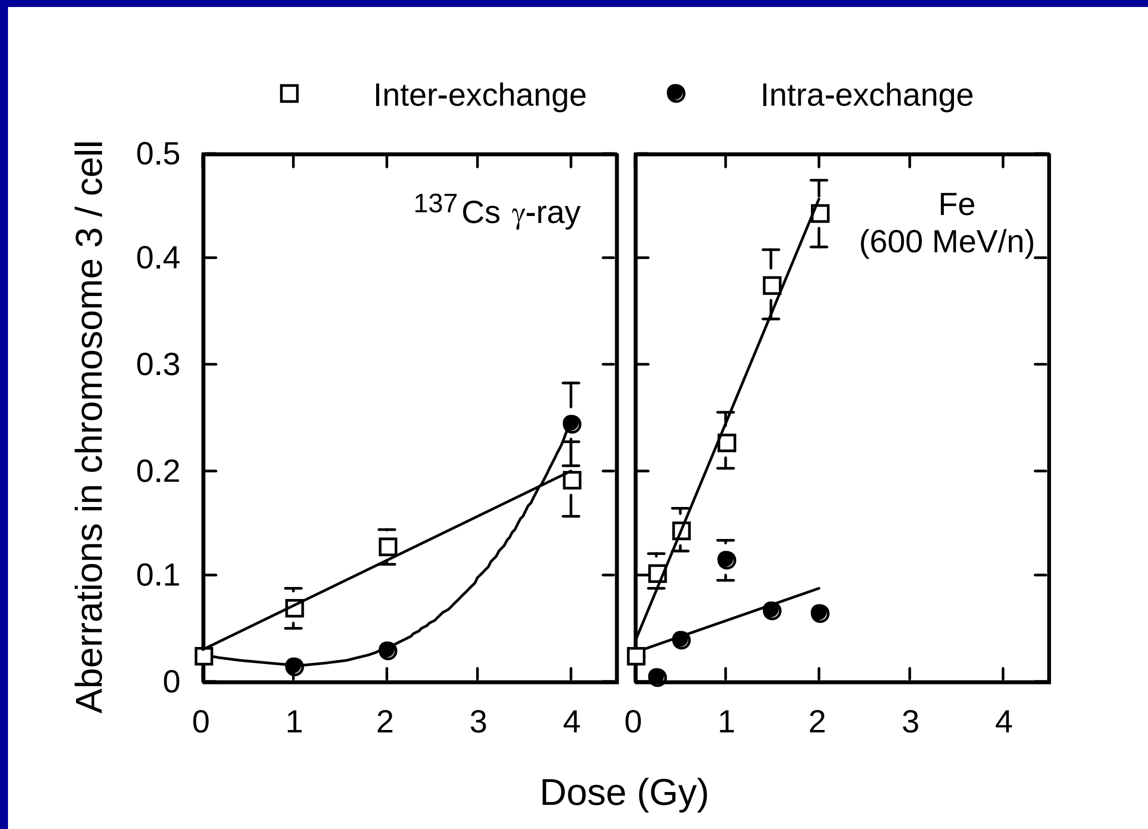
Radiation	Dose (Gy)	Harvest method	F ratio
γ ray	2	PCC	15.3±6.3
γ ray	2	Meta	12.5±5.9
γ ray	5	PCC	8.2±2.0
γ ray	5	Meta	9.1±2.5
1 GeV/u Fe	3	PCC	5.2±0.9
1 GeV/u Fe	3	Meta	9.1±2.2

Wu, George, Kawata, Willingham and Cucinotta, Rad. Res. 2001

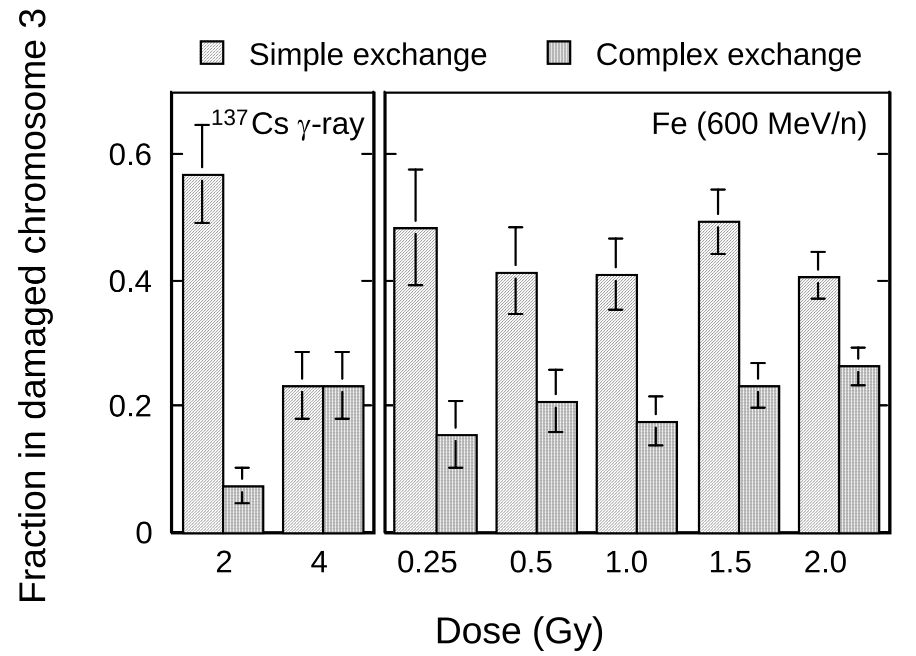
mBAND analysis

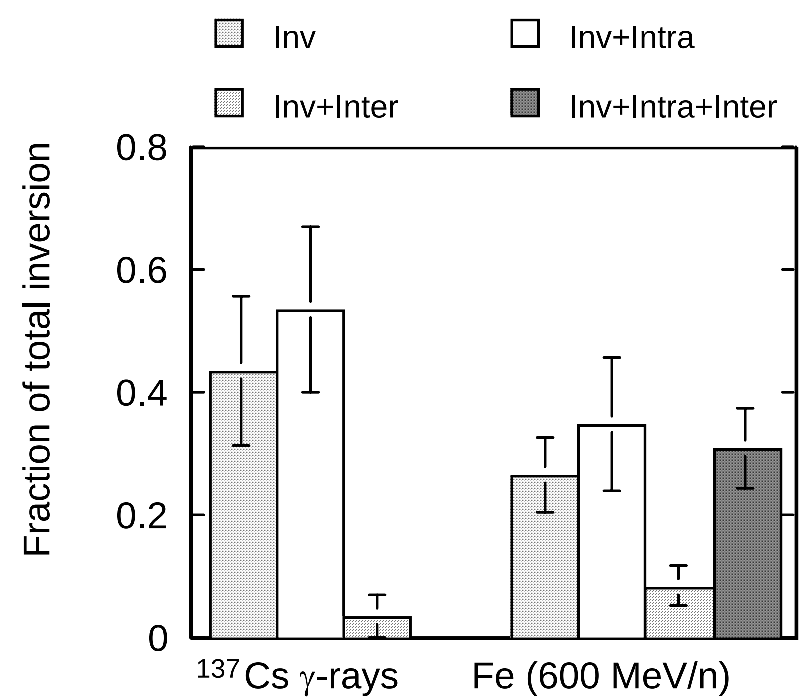


Inter- vs. intra chromosome exchanges (mBAND)



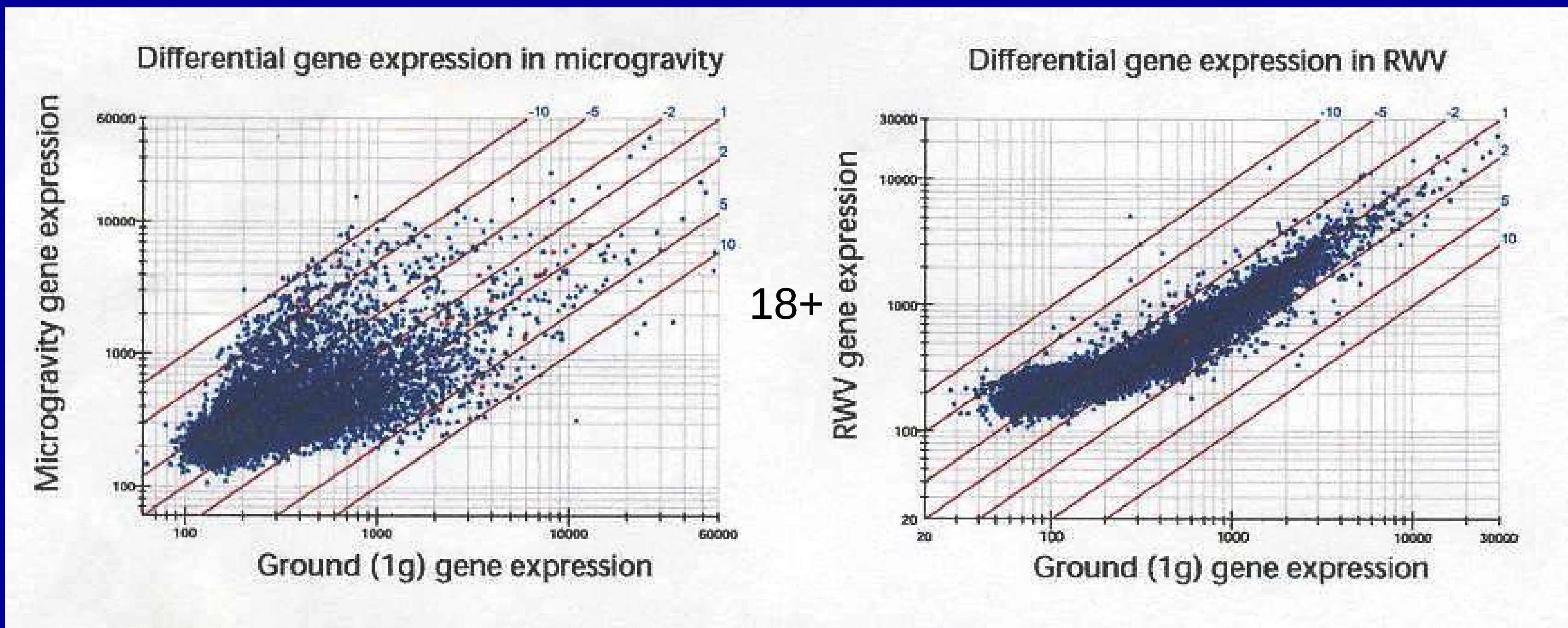
mBAND analysis





Most inversions were involved with other inter- and/or intra-chromosome rearrangements

Do spaceflight factors alter the cellular response to radiation exposure?



Hammond et al. Nature Medicine 1999

Chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated in vitro with low-LET radiation (Wu et al. Phys. Med. 2001)

Nission: STS-103

Duration: 8 days

Blood draw schedule:

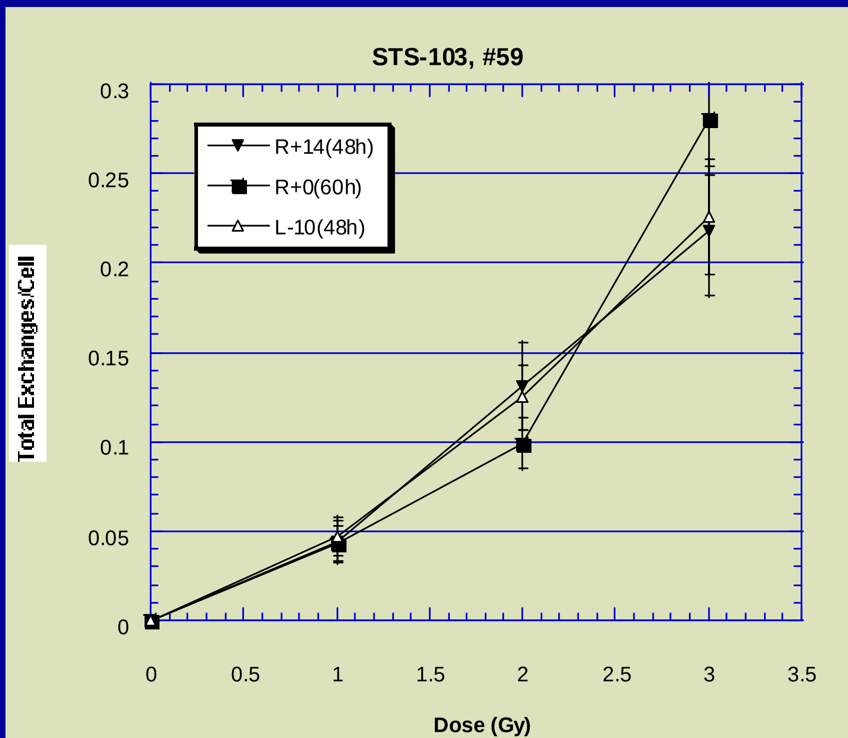
- 10 days before launch, JSC, kept at 4 C for 1 day before exposure
- 0 days after landing, KSC, kept at 4 C and received next day. Kept at 4 C before exposure
- 14 days after landing, KSC, kept at 4 C for 1 day before exposure

Irradiation: Whole blood was irradiated to gamma rays

Procedure: Whole blood was stimulated to grow with PHA in growth medium and chromosomes were collected following standard procedures.

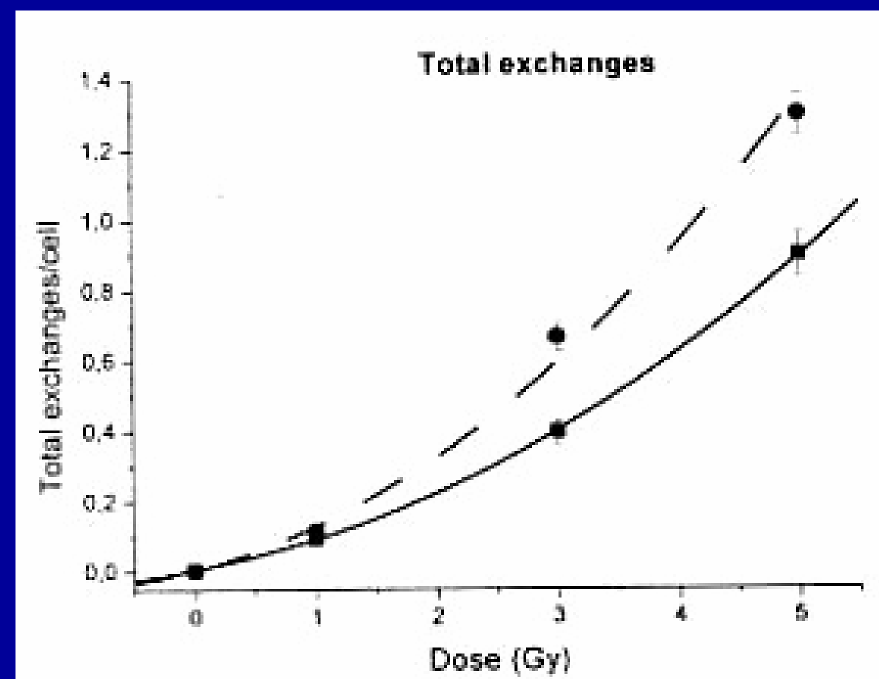
Chromosome analysis: Chromosomes #1 and #5 were painted.

Do spaceflight factors alter the cellular response to radiation exposure?



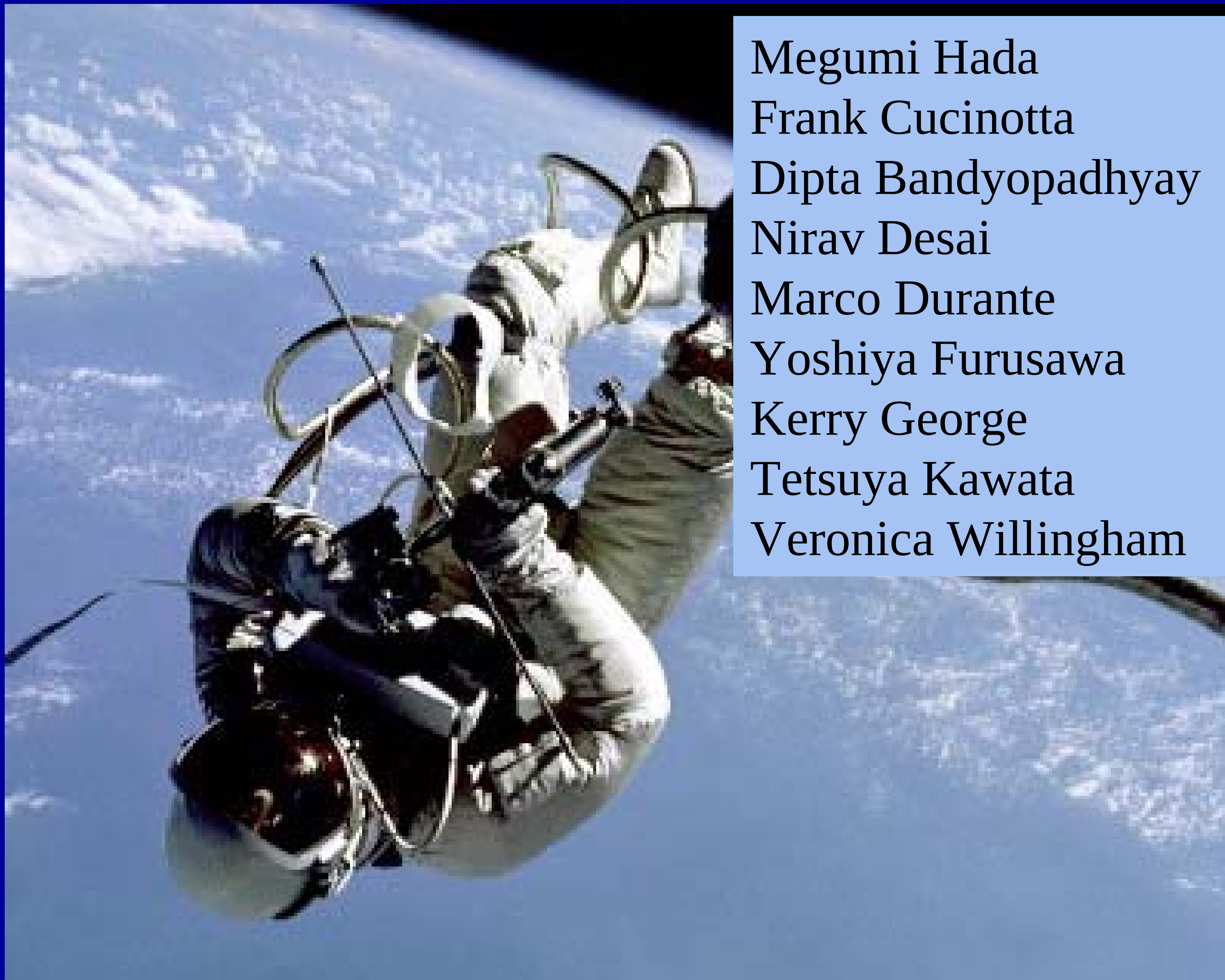
Wu, George, Willingham and Cucinotta, Physica Medica 2001

Greco et al. Adv. Space Res. 2003



Summary

- FISH, mFISH, mBAND, telomere and centromere probes have been used to study chromosome aberrations induced in human cells exposed to low- and high-LET radiation in vitro
- High-LET induced damages are mostly a single track effect
- Unrejoined chromosome breaks (incomplete exchanges) and complex type aberrations were higher for high-LET
- Biosignatures may depend on the method the samples are collected
- Recent mBAND analysis has revealed more information about the nature of intra-chromosome exchanges
- Whether space flight/microgravity affects radiation-induced chromosome aberration frequencies is still an open question.



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